EXHIBIT M

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Alkylation of Nucleic Acids and Metabolism of Small Doses of Dimethylnitrosamine in the Rat[1]

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ABSTRACT

Small doses (<100 μg/kg) of dimethylnitrosamine were metabolized very rapidly in the rat so that production of 7-methylguanine in liver DNA was virtually complete within 15 min. The amount of O^6-methylguanine found in DNA at this time was only 40% of that expected from the amount of 7-methylguanine found and declined rapidly over a further 3 hr, indicating that it was removed very rapidly from liver DNA. Dimethyl- and diethylnitrosamine were absorbed very rapidly from the upper part of the small intestine ($t½$, <3 min) but quite slowly ($t½$, >60 min) from the stomach. Since the latter was slower than the rate of metabolism after p.o. administration, the majority of uptake appears to be via the intestine. When the nitrosamine was administered p.o. in a mixture with a high fat content, the rate of reaction with liver DNA decreased, but the final extent of production of 7-methylguanine was the same, consistent with a reduction in the rate of absorption due to a decrease in the rate of gastric emptying.

The amount of 7-methylguanine formed in kidney DNA following administration of dimethylnitrosamine by i.v. injection was about 10 times less than the amount formed in liver DNA, irrespective of the dose over a range of 1 μg/kg to 10 mg/kg. A similar ratio was found after p.o. administration of doses of 5 to 10 mg/kg, but at doses of 1 mg/kg or below, there was much less production of 7-methylguanine in the kidney. These results suggest that, at the lower doses, the dimethylnitrosamine absorbed from the intestine into the portal blood supply is metabolized sufficiently rapidly by the liver that little becomes available for metabolism in the kidney. Removal of O^6-methylguanine from the renal DNA was slower than from the liver DNA, so that shortly after i.v. administration, the amount present in the kidney was more than half that in liver. Very little O^6-methylguanine was formed in the kidney DNA when doses of less than 50 μg/kg were given by p.o. administration. These results indicate the importance of the route of administration in determining the interaction of dimethylnitrosamine with various organs and suggest that the formation of alkylated bases in extrahepatic tissues may depend on the dose and on the rate of absorption of the carcinogen. The very high activity of the liver in removing O^6-methylguanine from DNA may provide a protective mechanism against tumor initiation.

INTRODUCTION

Dimethylnitrosamine is activated by oxidative metabolism to form an unstable hydroxylated derivative which decomposes to yield a highly reactive alkylating agent (10, 27). Such metabolism is obligatory for the carcinogenic action of the nitrosamine; and because of the instability of the metabolic product, only those tissues possessing the capacity to activate the compound are at risk for tumor development (3, 16, 17). The capacity to metabolize dimethylnitrosamine is greatest in the liver, but other tissues are able to activate it (3, 10, 16, 17, 21, 27). The rate of metabolism of dimethylnitrosamine after administration of large (>10 mg/kg) doses to rats was measured by Heath (8) and by Swann and McLean (34), who found that the compound was metabolized at a rate of about 5 mg/hr/kg. Wishnok *et al.* (35) reported that the disappearance of a 3-mg/kg dose of dimethylnitrosamine from the blood followed first-order kinetics. However, very rapid metabolism of small doses (<1 mg/kg) of dimethylnitrosamine was suggested in recent studies of the alkylation of rat liver and kidney DNA (2, 26). The disappearance of 1.67-μg/kg doses from mice was complete within 10 min (30). This decline was observed by measuring the concentration of dimethylnitrosamine in the blood or the whole animal at various times. Disappearance of the compound could, therefore, occur either by metabolism or by excretion into urine or expired air. In the present study, the rate of decomposition of dimethylnitrosamine has been estimated by measurement of the formation of 7-methylguanine in cellular DNA. 7-Methylguanine is the major product of the reaction of the alkylating species derived from dimethylnitrosamine with DNA and is removed from the DNA at a fairly slow rate (12-14, 18, 23, 31) which is insignificant over the time period of the present experiments. Therefore, the appearance of this product indicates the rate of conversion of the nitrosamine to the ultimate carcinogenic agent. At the same time, the amount of O^6-methylguanine present in the DNA was measured. This product, which may play a critical role in tumor initiation, can be removed enzymatically from liver DNA at a rapid rate (5, 20, 22-26, 28), but there have been only limited studies of its level at very short times after administration of the carcinogen (27, 28, 33).

MATERIALS AND METHODS

Chemicals. [^{3}H]Dimethylnitrosamine (2.96 or 3.49 Ci/mmol), [1-^{14}C]diethylnitrosamine (13.5 mCi/mmol), and [^{14}C]dimethylnitrosamine (26 mCi/mmol) were purchased from New England Nuclear, Boston, Mass. Unlabeled dimethylnitrosamine was redistilled from material obtained from Aldrich Chemical Company, Milwaukee, Wis. Marker methylated bases were obtained as described previously (24).

Administration of Dimethylnitrosamine. A solution in 0.9% (w/v) NaCl was used for i.p. or i.v. injections. A solution in water was used for p.o. administration. The specific radioactivity was adjusted by addition of unlabeled dimethylnitrosamine

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and is given in the legends. The volume administered was between 0.3 and 0.6 ml for i.p. and 0.1 to 0.3 ml for i.v. injections, which were given into the tail vein under ether anesthesia. The volume for p.o. treatment was about 1 ml. When the effects of fat on dimethylnitrosamine absorption and metabolism were tested, the rats received either 1 ml of olive oil or 1 ml of water in addition to the dose of labeled nitrosamines. All administrations of the carcinogen were carried out at about 9 a.m., and rats were deprived of food for 18 hr prior to treatment. Female Sprague-Dawley rats weighing about 200 to 250 g were used. The rats were maintained under a controlled 12-hr light, 12-hr dark cycle and were allowed free access to water and rat chow prior to the experiments.

Preparation and Analysis of DNA. The rats were killed by cervical dislocation, and liver and kidneys were rapidly removed and frozen in liquid N_2. DNA was isolated from the frozen tissues by phenol extraction (24, 28). The dried DNA was hydrolyzed in 0.1 N HCl for 30 min at 37°, and the purines were separated on columns of Sephadex G10 and quantitated as described previously (24, 28). Results were expressed as μmol of 7-methylguanine or O^6-methylguanine per mol of guanine. At least 100 cpm above background were present in the peaks corresponding to the methylated base.

Uptake of Nitrosamines from Gastrointestinal Tract. Absorption of [^{3}H]dimethylnitrosamine or [^{14}C]diethylnitrosamine from the stomach was measured as follows. The stomach was ligated at the pyloric sphincter, and 10 ml of water containing 0.2 μg of [^{3}H]dimethylnitrosamine or [^{14}C]diethylnitrosamine (about 50,000 cpm) were introduced. Samples were removed at various times, and the radioactivity remaining was determined. [^{14}C]- or [^{3}H]Sorbitol (as appropriate) was also placed in the solution to provide a nonabsorbed control which could be used to correct for changes in the volume of gastric fluid. Absorption from the intestine was measured by ligating a loop from the pylorus to about 15 cm further down under anesthesia with sodium pentobarbitol (6). The nitrosamines were introduced into the loop in the total volume of about 1 ml of 0.9% NaCl solution, and at various times, the entire contents were assayed for radioactivity using separate rats for each time point.

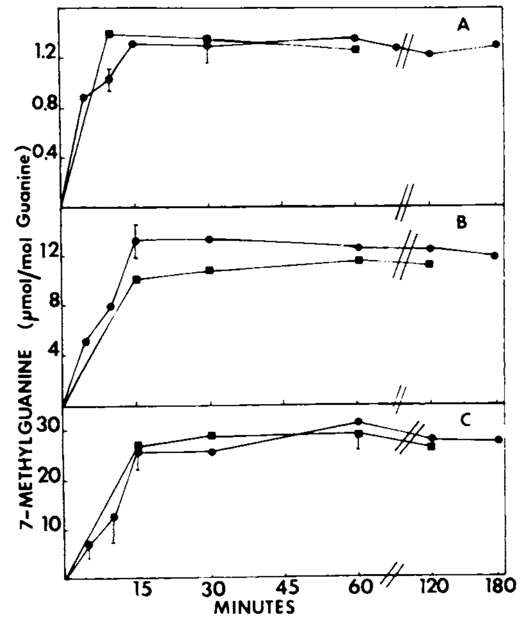

Chart 1. Presence of 7-methylguanine in liver DNA after treatment with dimethylnitrosamine. [^{3}H]Dimethylnitrosamine was administered at a dose of 5 μg/kg (*A*), 50 μg/kg (*B*), or 100 μg/kg (*C*) by p.o. (■) or i.p. (●) administration, and the amount of 7-methylguanine was measured at the time shown. *Bars*, S.E.

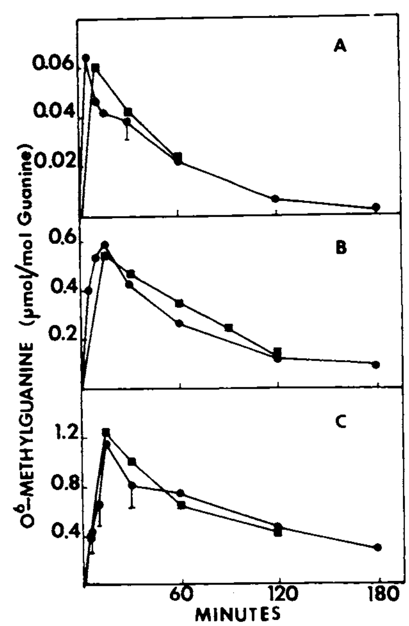

Chart 2. Presence of O^6-methylguanine in liver DNA after treatment with dimethylnitrosamine. Details were as in Chart 1.

RESULTS

Chart 1 indicates the time course of production of 7-methylguanine in liver DNA after treatment with 5-, 50-, or 100-μg/kg doses of dimethylnitrosamine given by i.p. injection. It can be seen that formation of the adduct is complete within about 15 min and that at least 50% of the maximal value was reached within 10 min. Values for 7-methylguanine formation in liver DNA are also given in Chart 1 after treatment with dimethylnitrosamine by p.o. administration. Again, alkylation reached virtually (>85%) maximal levels within 15 min. These results indicate that the ability of the liver to metabolize the carcinogen is sufficiently great that clearance is very rapid.

The rate of removal of 7-methylguanine from liver DNA is sufficiently slow (18, 23) to prove negligible over the 3-hr time course covered by Chart 1, and as can be seen, there was no significant change in the level over the period from 30 to 180 min. This was not the case with O^6-methylguanine, another alkylation product. As shown in Chart 2, this product also reached a maximum within the first 15 min after the carcinogen was administered and then declined rapidly. This finding is in agreement with recent reports of an active enzyme system in liver cells capable of catalyzing removal of O^6-methylguanine from DNA (5, 18, 22-26, 28, 33). In fact, even the maximal level of O^6-methylguanine found in the present experiments within the first 15 min of administration of the carcinogen amounts to only 36 to 44% of that expected, based on the amount of 7-methylguanine (12, 13, 18, 23, 28). Therefore, it appears that over half of this product is removed within the 15-min period. As found for 7-methylguanine, the levels of O^6-methylguanine observed after p.o. administration of dimethylnitrosamine were in close agreement to those found after i.p. injection.

After p.o. administration of dimethylnitrosamine, it is necessary for the compound to be absorbed from the gastrointestinal tract in order to be metabolized by the liver, and since it is shown in Chart 1 that this metabolism is complete within a few min, such uptake must be very rapid. Table 1 shows the absorption of dialkylnitrosamine from the rat stomach and from the upper part of the small intestine. It can be seen that uptake of dimethylnitrosamine and diethylnitrosamine from the stomach was quite slow with a half-life of about 1 hr. In contrast, uptake from the intestine was very rapid ($t½$ <3 min). These results are in agreement with those of others using different strains of rat (6, 7) and indicate clearly that the dimethylnitrosamine administered by p.o. administration is absorbed from the intestine rather than the stomach. If this is the case, then factors which slow the rate of gastric emptying should slow the appearance of methylated bases in the liver DNA. As shown in Table 2, this is indeed the case. The ratio of methylation seen after administration of dimethylnitrosamine in water to that seen after administration in a mixture containing a large amount of fat is significantly greater than 1.0 at early times, but it becomes unity as time increases, and methylation in the fat-treated rats continued to increase while that in the controls remained constant.

Formation of 7-methylguanine and O^6-methylguanine was also measured in the kidney, an extrahepatic organ known to metabolize dimethylnitrosamine (2, 21, 27, 34), and compared to that found in the liver. Table 3 shows the amount of 7-methylguanine formed in these tissues after p.o. or i.v. administration of the carcinogen. It can be seen that, after i.v. injection, the amount of 7-methylguanine found in the DNA of both tissues was proportional to the dose and that there was about 9 times more 7-methylguanine in the liver DNA than in the kidney, which is consistent with the substantially greater activating capacity in the liver. When the dimethylnitrosamine was given via p.o. administration, there was little difference in the amount of 7-methylguanine found in the liver DNA, but that found in the kidney DNA was strikingly decreased at all doses of 1 mg/kg and below. Only with doses of more than 1 mg/kg was the same ratio of reaction with the liver and kidney seen when dimethylnitrosamine was given by either route.

The much lower extent of reaction with kidney DNA following small p.o. doses of dimethylnitrosamine was also seen when O^6-methylguanine was measured. [^{3}H]Dimethylnitrosamine of very high specific activity (3.49 Ci/mmol) was used in this experiment in order to measure the amount of O^6-methylguanine present after doses of less than 1 mg/kg (Table 4). It can be seen that the amount of O^6-methylguanine in the liver did not vary with the route of administration and ranged from 0.01 µmol/mol guanine with 1 µg/kg, which is 18% of that expected on the basis of the 7-methylguanine, to 0.36 µmol/mol guanine after 100 µg/kg, which is about 8% of that expected (Table 4). The amount of O^6-methylguanine in the kidney DNA was much greater after i.v. administration, as expected from the results with 7-methylguanine. The removal of O^6-methylguanine from the renal DNA was considerably slower than from the liver, and the amounts found ranged from 85 to 45% of that produced. Because of the more complete removal of O^6-methylguanine from the liver DNA, the liver:kidney alkylation ratio at this site was only about 1.8 in the rats given the carcinogen by i.v.

Table 1

Uptake of dimethylnitrosamine and diethynitrosamine from the rat stomach and small intestine

Uptake was measured as described under "Materials and Methods" using 0.2 to 5 µg of the nitrosamines. There was no significant difference in the rate of absorption between different doses in this range, and the results are expressed as the mean percentages of the initial dose remaining at the time shown.

Time (min)	Amount remaining in intestine (%)		Amount remaining in stomach (%)	
	Dimethylnitrosamine	Diethylnitrosamine	Dimethylnitrosamine	Diethylnitrosamine
0	100	100	100	100
2	58	55		
3	46	42		
7	20	20		
9	12	11		
12	6	5		
15	3	2	90	82
30	<1	<1	76	68
60	<1	<1	62	50
90	<1	<1	52	38

Table 2

Ratio of alkylation of liver DNA after p.o. administration of dimethylnitrosamine in water or in lipid emulsions

The dimethylnitrosamine was administered by stomach tube in 2 ml of water or 2 ml of a 1:1 water:olive oil mixture. At the times shown, the livers were removed, and the alkylation of DNA at position 7 of guanine was determined. Results are shown as the ratio of alkylation in the rats given the nitrosamine in water alone to that given in the presence of fat. The values given are derived from the mean of 3 separate rats for each dose and time. Individual values agreed within ± 15%.

Time (min)	Dose of dimethylnitrosamine (µg/kg)	Ratio of 7-methylguanine in DNA after administration in water to 7-methylguanine in DNA after administration plus lipid
10	5	1.50
10	25	1.67
30	5	1.16
30	25	1.33
30	760	1.27
30	1520	1.32
60	5	0.97
60	25	1.05
60	760	1.01
60	1520	0.94

Table 3

Formation of 7-methylguanine in liver and kidney DNA following administration of dimethylnitrosamine by p.o. or i.v. administration

Dimethylnitrosamine was administered by p.o. or i.v. injection as described under "Materials and Methods," and the extent of alkylation of DNA at position 7 of guanine was measured 3 hr later. Results shown are the mean of 3 estimations which agreed within ± 15%.

Dose (µg/kg)	Route of administration	7-Methylguanine in DNA (µmol/mol)		Ratio of alkylation (liver:kidney)
		Liver	Kidney	
1	i.v.	0.45	0.05	9
5	i.v.	2.4	0.22	11
10	i.v.	4.3	0.46	9
50	i.v.	20	2.5	8
100	i.v.	42	4.9	9
1,000	i.v.	320	34	9
5,000	i.v.	1,390	145	10
10,000	i.v.	3,110	342	9
1	p.o.	0.6	<0.005	<120
5	p.o.	2.5	0.02	125
10	p.o.	5.0	0.06	83
50	p.o.	24	0.42	57
100	p.o.	47	1.3	36
1,000	p.o.	292	16.2	18
5,000	p.o.	1,510	150	10
10,000	p.o.	3,280	358	9

Table 4

Formation of O^6-methylguanine in liver and kidney DNA following administration of dimethylnitrosamine by p.o. or i.v. administration

Dimethylnitrosamine was administered by p.o. or i.v. injection, and the amount of O^6-methylguanine present in the liver and kidney DNA was measured 60 min later (for the doses of 1 to 50 μg/kg) or 3 hr later (for 100 μg/kg). The amount of O^6-methylguanine given is the mean of 3 estimations which agreed within ± 20%. The amount formed was calculated by multiplying the amount of 7-methylguanine in Table 3 by 0.11, since methylation at the O^6 site occurs at about 11% of methylation at position 7 (13, 18, 23, 28).

Dose (μg/kg)	Route of administration	O^6-Methylguanine in DNA (μmol/mol)		Ratio of alkylation (liver:kidney)
		Liver	Kidney	
1	i.v.	0.010 (20)[a]	0.047 (85)	2.1
5	i.v.	0.034 (13)	0.018 (74)	1.9
10	i.v.	0.061 (13)	0.031 (61)	2.0
50	i.v.	0.26 (12)	0.15 (55)	1.7
100	i.v.	0.36 (8)	0.24 (45)	1.5
1	p.o.	0.009 (14)	<0.0001	>90
5	p.o.	0.038 (14)	0.0015 (68)	25
10	p.o.	0.07 (13)	0.004 (61)	18
50	p.o.	0.28 (11)	0.040 (86)	7.0
100	p.o.	0.40 (8)	0.085 (59)	4.7

[a] Numbers in parentheses, percentage of the amount formed.

injection. However, the ratio was much greater after p.o. administration because of the low level of interaction with the kidney with this route of administration.

DISCUSSION

The present results confirm that metabolism of small doses of dimethylnitrosamine occurs very rapidly (30) and show that alkylation of DNA is complete within a few min of exposure via either p.o. or parenteral administration. There is substantial evidence suggesting that the formation of O^6-methylguanine in DNA and its persistence through cell division are important in tumor initiation by nitrosamines (5, 13, 14, 17, 18, 23). The very rapid removal of O^6-methylguanine from liver DNA when small doses of dimethylnitrosamine are given may provide an important protective mechanism against carcinogenesis. It should be noted, however, that, as shown in Chart 2 and Table 4, there appears to be a small amount of the O^6-methylguanine produced which is somewhat more resistant to removal. Previous publications in which measurements were made over a longer period of time also indicated a resistant fraction (22, 26–28). It is quite possible that this may arise from the alkylation of DNA in some of the nonhepatocyte cells in the liver. It is known that tumors can arise from such other cell types in response to dimethylnitrosamine (3, 9, 16, 17), and recently, it has been reported that O^6-methylguanine produced in nonparenchymal cells by 1,2-dimethylhydrazine is much more persistent than in the hepatocytes (15). Even within the hepatocytes themselves, there may be variations in the ability to remove O^6-methylguanine and in the extent to which they become alkylated due to their position within the liver lobules. In any event, it should be stressed that, while a rapid DNA repair reaction like the removal of O^6-methylguanine from DNA may protect against carcinogenesis, there would always be some risk of tumor initiation due to the time in which the O^6-methylguanine is present. On prolonged treatment even with low levels of the carcinogen, some tumors would be expected. When the repair system becomes overloaded and when the potentially harmful product builds up as the level of exposure is increased, a rise in the tumor incidences greater than the simple increase in the dose would be produced (26). Chronic treatment with dimethylnitrosamine may actually induce the activity of the O^6-methylguanine removal system (19, 20, 26, 33) and lead to an even more rapid loss of this product from DNA than that observed in the presence of single-dose experiments. Such an effort would increase the dose which could be tolerated before the build-up of O^6-methylguanine.

The kidney is less able than the liver to remove O^6-methylguanine from DNA after large extents of alkylation by high doses of dimethylnitrosamine (22, 23) and after small extents of alkylation produced by small doses (Table 4; Ref. 28). Therefore, it may be more at risk for tumor initiation. This is the case after large single doses of dimethylnitrosamine which produce exclusively kidney tumors in the rat (22), but liver tumors and no kidney tumors were seen after chronic exposure in the diet (3, 9, 16, 17). The results in the present paper, which are in agreement with those of Diaz Gomez *et al.* (2), provide an explanation for this in that there is very little interaction with the kidney when the dimethylnitrosamine is administered p.o. In our experiments which used a much higher radiospecific activity of the dimethylnitrosamine and, therefore, had a much greater sensitivity, we were able to detect 7-methylguanine in the kidney after doses of 40 μg/kg or lower for which Diaz Gomez *et al.* (2) were unable to find any reaction. Also, we were able to measure O^6-methylguanine in the kidney DNA at all doses except after 1 μg/kg given p.o. Nevertheless, there was much less of this product present in the kidney DNA than in the liver after p.o. administration of doses of dimethylnitrosamine of 100 μg/kg or below. When the carcinogen was given i.v., there was much less difference between the 2 organs, but no experiments in which tumor incidence was examined after chronic administration via this route have been reported.

It is apparent from work described in this paper and from reports by others who have also observed a slow rate of uptake of dimethylnitrosamine from the stomach and a much more rapid rate from the intestine (6, 7, 29) that the bulk of the uptake of the nitrosamine must take place from the intestine. Therefore, the rate of appearance of the nitrosamine in the portal blood supply and hence its metabolism by the liver may depend on the rate of gastric emptying. Factors influencing gastric emptying may, therefore, slow the rate of absorption and metabolism. Such a factor would be a high lipid content, and, as shown in Table 2, this does retard, but not ultimately prevent, the alkylation of liver DNA. It is also possible that the presence of fat is able to retard the uptake of the nitrosamine directly, and some evidence for this has been published (1). As discussed above, the efficient metabolism by the liver of low levels of dimethylnitrosamine may protect other organs such as the kidney from reacting with the carcinogen. It is possible that alterations of the rate of absorption from the gastrointestinal tract might alter the dose below which substantial hepatic clearance can take place. In this way, dietary factors could significantly alter the interaction with organs, such as the kidney and the lung, which are sensitive to carcinogenesis by dimethylnitrosamine. Further experimental investigation of this possibility is obviously required.

Several groups have reported finding small but significant quantities (of the order of 0.5 to 1.2 ng/ml) of dimethylnitrosamine in human blood (4, 11, 36). Although some increase was detected after a meal in the earlier paper (4), this was not

confirmed in the later studies (11, 36). However all of these groups found dimethylnitrosamine in the peripheral blood. The rate of clearance of dimethylnitrosamine by the human liver should be similar to that in the rat, since it is known that human liver slices metabolize dimethylnitrosamine at about the same rate as the rat (21). The presence of constant levels of dimethylnitrosamine in the blood indicates steady production of the carcinogen, and the rapid clearance by metabolism in the liver should be taken into account in calculating the possible daily human exposure level. Finally, recent studies by Sklar and Strauss (32) have shown that some human lymphoblastoid cell lines are very active in removing O^6-methylguanine from DNA. Also, human liver extracts were found to be 6 to 10 times more active than rat liver extracts in catalyzing the removal of O^6-methylguanine from DNA *in vitro.*[3] These results suggest that the ability of human liver to carry out repair of these lesions may be even greater than that of the rat.

REFERENCES

1. Agrelo, C., Phillips, J. C., Lake, B. G., Longland, R. C., and Gangolli, S. D. Studies on the gastrointestinal absorption of *N*-nitrosamines: effect of dietary constituents. Toxicology, *10:* 159-167, 1978.
2. Diaz Gomez, M. I., Swann, P. F., and Magee, P. N. The absorption and metabolism in rats of small oral doses of dimethylnitrosamine. Biochem. J., *164:* 417-500, 1977
3. Druckrey, H., Preussman, R., Ivankovic, S., and Schmähl, D. Organotrope carcinogene Wirkungen bei 65 verschiedenen N. Nitroso-Verbindungen an BD-Rattan. Z. Krebsforsch., *69:* 103-201, 1967.
4. Finé, D. H., Ross, R., Rounbehler, D. P., Silvergleid, A., and Song, L. Formation *in vivo* of volatile *N*-nitrosamines in man after ingestion of cooked bacon and spinach. Nature (Lond.), *265:* 753-755, 1977.
5. Goth, R., and Rajewsky, M. F. Persistence of O^6-ethylguanine in rat brain DNA. Proc. Natl. Acad. Sci. U. S. A., *71:* 639-643, 1974.
6. Hashimoto, S., Yokukura, T., Kawai, Y., and Mutai, M. Dimethylnitrosamine formation in the gastrointestinal tract of rats. Food Cosmet. Toxicol., *14:* 553-556, 1976.
7. Heading, C. E., Phillips, J. C., Lake, B. G., Gangolli, S. D., and Lloyd, A. G. Some aspects of the metabolism of dimethylnitrosamine in the rat. Biochem. Soc. Trans., *2:* 607-611, 1974.
8. Heath, D. F. The decomposition and toxicity of dialkylnitrosamines in rats. Biochem. J., *85:* 72-81, 1962.
9. IARC. Monographs on the evaluation of the carcinogenic risk of chemicals to International Agency for Research on Cancer, Vol. 17. *In:* Some *N*-Nitroso-Compounds. Lyon, France: International Agency for Research on Cancer, 1978.
10. Lai, D. Y., and Arcos, J. C. Dialkylnitrosamine bioactivation and carcinogenesis. Life Sci., *27:* 2149-2165, 1980.
11. Lakritz, L., Simenhoff, M. L., Dunn, S. R., and Fiddler, W. *N*-Nitrosodimethylamine in human blood. Food Cosmet. Toxicol., *18:* 77-79, 1980.
12. Lawley, P. D. Carcinogenesis by alkylating agents. ACS (Am. Chem. Soc.) Monogr., *173:* 83-244, 1976.
13. Lawley, P. D. Methylation of DNA by carcinogens. Some applications of chemical analytical methods. *In:* R. Montesano, H. Bartsch, and L. Tomatis (eds.), Screening Test in Chemical Carcinogenesis, IARC Scientific Publication 12, pp. 181-210. Lyon, France: International Agency for Research on Cancer, 1976.
14. Lawley, P. D. DNA as a target of alkylating carcinogens. Br. Med. Bull., *36:* 19-24, 1980.
15. Lewis, J. G., and Swenberg, J. A. Differential repair of O^6-methylguanine in DNA of rat hepatocytes and nonparenchymal cells. Nature (Lond.), *288:* 185-187, 1980.
16. Magee, P. N., and Barnes, J. M. Carcinogenic nitroso compounds. Adv. Cancer Res., *10:* 163-246, 1967.
17. Magee, P. N., Montesano, R., and Preussman, R. *N*-Nitroso compounds and related carcinogens. ACS (Am. Chem. Soc.) Monogr., *173:* 491-625, 1976.
18. Margison, G. P., and O'Connor, P. J. Nucleic acid modification by *N*-nitroso compounds. *In:* P. L. Grover (ed.), Chemical Carcinogens and DNA, pp. 111-159. West Palm Beach, Fla.: CRC Press, 1979.
19. Montesano, R., Brésil, H., and Margison, G. P. Increased excision of O^6-methylguanine from rat liver DNA after chronic administration of dimethylnitrosamine. Cancer Res., *39:* 1798-1802, 1979.
20. Montesano, R., Brésil, H., Planche-Mortel, G., Margison, G. P., and Pegg, A. E. Effect of chronic treatment of rats with dimethylnitrosamine on the removal of O^6-methylguanine from DNA. Cancer Res., *40:* 452-458, 1980.
21. Montesano, R., and Magee, P. N. Comparative metabolism *in vitro* of nitrosamines in various animal species including man. *In:* R. Montesano and L. Tomatis (eds.), Chemical Carcinogenesis Essays, IARC Scientific Publication 10, pp. 39-56. Lyon, France: International Agency for Research on Cancer, 1974.
22. Nicoll, J. W., Swann, P. F., and Pegg, A. E. Effect of dimethylnitrosamine on persistence of methylated guanines in rat liver and kidney DNA. Nature (Lond.), *256:* 261-262, 1975.
23. Pegg, A. E. Formation and metabolism of alkylated purines: possible role in carcinogenesis by *N*-nitroso compounds and akylating agents. Adv. Cancer Res., *25:* 195-269, 1977.
24. Pegg, A. E. Alkylation of rat liver DNA by dimethylnitrosamine: effect of dosage on O^6-methylguanine levels. J. Natl. Cancer Inst., *58:* 681-687, 1977.
25. Pegg, A. E. Enzymatic removal of O^6-methylguanine from DNA by mammalian cell extracts. Biochem. Biophys. Res. Commun., *84:* 166-173, 1978.
26. Pegg, A. E. Formation and subsequent repair of alkylation lesions in tissues of rodents treated with nitrosamines. Arch. Toxicol., (Suppl. 3)*:* 55-58, 1980.
27. Pegg, A. E. Metabolism of *N*-nitrosodimethylamine. *In:* R. Montesano, H. Bartsch, and L. Tomatis (eds.), Molecular and Cellular Aspects of Carcinogen Screening Tests, IARC Scientific Publication 27, pp. 3-22. Lyon, France: International Agency for Research on Cancer, 1980.
28. Pegg, A. E., and Hui, G. Formation and subsequent removal of O^6-methylguanine from DNA in rat liver and kidney after small doses of dimethylnitrosamine. Biochem. J., *173:* 739-748, 1978.
29. Phillips, J. C., Lake, B. G., Heading, C. E., Gangolli, S. D., and Lloyd, A. G. Studies on the metabolism of dimethylnitrosamine in the rat. I. Effect of dose, route of administration, and sex. Food Cosmet. Toxicol., *13:* 203-209, 1975.
30. Rounbehler, D. P., Ross, R., Fine, D. H., Iqbal, Z. M., and Epstein, S. S. Quantitation of dimethylnitrosamine in the whole mouse after biosynthesis *in vivo* from trace levels of precursors. Science (Wash. D. C.), *197:* 917-918, 1977.
31. Singer, B. Sites in nucleic acids reacting with alkylating agents of different carcinogenicity or mutagenicity. J. Toxicol. Environ. Health, *2:* 1279-1295, 1977.
32. Sklar, R., and Strauss, B. Removal of O^6-methylguanine from DNA of normal and xeroderma pigmentosum-derived lymphoblastoid lines. Nature (Lond.), *289:* 417-420, 1981.
33. Swann, P. F., and Mace, R. Changes in O^6-methylguanine disappearance from rat liver DNA during chronic dimethylnitrosamine administration. Chem.-Biol. Interact., *31:* 239-245, 1980.
34. Swann, P. F., and McLean, A. E. M. Cellular injury and carcinogenesis. The effect of a protein-free, high carbohydrate diet on the metabolism of dimethylnitrosamine in the rat. Biochem. J., *124:* 283-288, 1971.
35. Wishnok, J. S., Rogers, A. E., Sanchez, O., and Archer, M. C. Dietary effects of the pharmacokinetics of three carcinogenic nitrosamines. Toxicol. Appl. Pharmacol., *43:* 391-398, 1978.
36. Yamamoto, M., Yamada, T., and Tanimura, A. Volatile nitrosamines in human blood before and after ingestion of a meal containing high concentrations of nitrate and secondary amines. Food Cosmet. Toxicol., *18:* 297-299, 1980.

[3] A. E. Pegg, M. Roberfroid, H. Brésil, A. Likhachev, and R. Montesano, unpublished observations.

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